

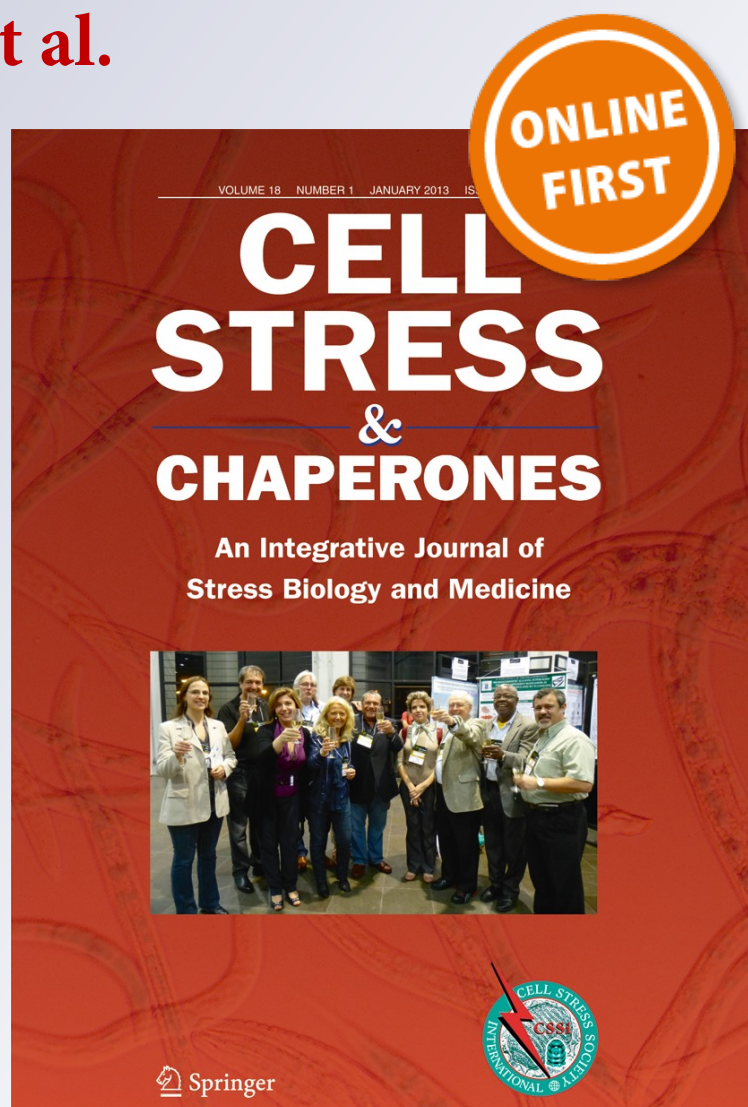
*In MMTV-Her-2/neu transgenic mammary tumors the absence of caveolin-1<sup>-/-</sup> alters PTEN and NHERF1 but not  $\beta$ -catenin expression*

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# In MMTV-Her-2/neu transgenic mammary tumors the absence of caveolin-1<sup>-/-</sup> alters PTEN and NHERF1 but not $\beta$ -catenin expression

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**Abstract** In a recent study, we have shown that in mammary tumors from mice lacking the *Cav-1* gene, there are alterations in specific heat shock proteins as well as in tumor development. With this in mind, we have now investigated other proteins in the same mammary mouse tumor model (Her-2/neu expressing mammary tumors from Cav-1 wild type and Cav-1 null mice), to further comprehend the complex tumor-stroma mechanisms involved in regulating stress responses during tumor development. In this tumor model the cancer cells always lacked of Cav-1, so the KO influenced the Cav-1 in the stroma. By

immunohistochemistry, we have found a striking co-expression of  $\beta$ -catenin and Her-2/neu in the tumor cells. The absence of Cav-1 in the tumor stroma had no effect on expression or localization of  $\beta$ -catenin and Her-2/neu. Both proteins appeared co-localized at the cell surface during tumor development and progression. Since Her-2/neu activation induces MTA1, we next evaluated MTA1 in the mouse tumors. Although this protein was found in numerous nuclei, the absence of Cav-1 did not alter its expression level. In contrast, significantly more PTEN protein was noted in the

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tumors lacking Cav-1 in the stroma, with the protein localized mainly in the nuclei. P-Akt levels were relatively low in tumors from both Cav-1 WT and Cav-1 KO mice. There was also an increase in nuclear NHERF1 expression levels in the tumors arising from Cav-1 KO mice. The data obtained in the MMTV-neu model are consistent with a role for Cav-1 in adjacent breast cancer stromal cells in modulating the expression and localization of important proteins implicated in tumor cell behavior.

**Keywords** Caveolin-1 · PTEN · NHERF1 ·  $\beta$ -Catenin · Breast cancer · Her-2/neu · Oncogene

## Abbreviation

Cav-1	Caveolin-1
EMT	Epithelial to mesenchymal transition
ER	Estrogen receptor
Her-2/neu	c-erbB-2
HSP	Heat shock protein
IHC	Immunohistochemistry
KO	Knockout
MMTV	Murine mammary tumor virus
MTA1	Metastasis associated protein 1
NHERF1	Na <sup>+</sup> /H <sup>+</sup> exchanger regulator factor 1
PR	Progesterone receptor
PTEN	Phosphatase and tensin homologue deleted on chromosome 10
WT	Wild type

## Introduction

In a previous study of human breast cancer samples, we reported that high levels of Cav-1 in stromal tissues surrounding the tumor were strongly associated with reduced metastasis and improved survival (Sloan et al. 2009). In this study, we also report (in a MMTV-c-neu transgenic mouse tumor model) that the onset of mammary tumors driven by specific expression of Her-2/neu is accelerated in mice lacking Cav-1<sup>-/-</sup>, supporting the concept that the presence of Cav-1 in the tumor microenvironment regulates the rate of tumor development.

We then hypothesize that since the loss of Cav-1 has been related to oxidative stress (Pavlidis et al. 2010) and that the heat shock proteins (HSPs) are induced by stress (Kampinga et al. 2009), the absence of Cav-1 is likely to initiate a stress response in the tumor cells. In fact, this was reported in a recent study where the levels of Hsp70 (HSPA) were almost double in Cav-1<sup>-/-</sup> tumors compared to Cav-1<sup>+/+</sup> tumors (Ciocca et al. 2012). In contrast, Hsp27/Hsp25 (HSPB1) levels were significantly lower in the Cav-1<sup>-/-</sup> tumors. Moreover, in mammary tumors from animals lacking Cav-1, we found a significant reduction in the extent of apoptosis, demonstrating that the

disruption of the *Cav-1* gene can cause alterations in specific HSPs as well as in tumor cell survival.

In the present study, using this unique tumor model (Her-2/neu expressing mammary tumors from Cav-1 wild type and Cav-1 null mice), we examined other proteins with the aim of advancing our understanding of the complexity of regulation of stress response and tumor development. We selected a series of proteins that are all mechanistically related with stress and/or heat shock protein response:  $\beta$ -catenin, MTA1, PTEN, Akt and NHERF1.

In human breast cancer cells and tissues,  $\beta$ -catenin interacts with Hsp27, Cav-1, and heat shock factor 1, interactions that may explain some of the molecular pathways that influence tumor cell survival and disease outcome (Fanelli et al. 2008). In addition, it has been shown previously that the simultaneous deregulation of both: (a) Wnt signaling through  $\beta$ -catenin and (b) Her-2/neu, cooperate to induce mammary gland tumors in transgenic mice (Schroeder et al. 2002).

MTA1 was selected because in human breast cancer, heregulin, which is an indirect activator of the Her-2/neu pathway, strongly induced MTA1/heat shock factor 1 complexes with a number of associated proteins, including histone deacetylases HDAC1, HDAC2 and Mi2, that are components of the NuRD co-repressor complex (Khaleque et al. 2008). These complexes participate in the repression of estrogen-dependent transcription and can explain, at least in part, the shorter disease-free survival and overall survival reported in breast cancer patients whose tumors co-express ERs and/or PRs with Her-2/neu (Ciocca et al. 2006).

PTEN is a tumor suppressor gene encoding an enzyme involved in the regulation of various cellular processes. The tumor suppressor function may be explained by its activity as a protein tyrosine phosphatase and as a phosphatidylinositol phosphate (PIP) phosphatase (Moncalero et al. 2011). The PI3K/Akt signaling pathway is negatively regulated by PTEN. Mutations, deletions or silencing of PTEN cause increases in the PI3K signal, which in turn stimulate downstream Akt signaling leading to promotion of growth factor-independent growth and increased cell invasion and metastasis (Hafsi et al. 2012). Activated Akt is a well-established survival factor, exerting anti-apoptotic activity by preventing the release of cytochrome C from mitochondria and inactivating Forkhead transcription factors (FKHR), which are known to induce the expression of genes that are critical for apoptosis (Fukunaga and Shioda 2009; Fiandalo and Kyprianou 2012). We have recent evidence to indicate that the down-regulation of Hsp27 (HSPB1) in MCF-7 human breast cancer cells induces up-regulation of PTEN and reduces p-Akt levels (Cayado-Gutiérrez et al. 2012).

Finally, we also analyzed the adaptor protein NHERF1, because of its important role in maintaining the integrity of cell–cell interactions and in stabilizing E-cadherin/ $\beta$ -catenin complexes (Kreimann et al. 2007). NHERF1 may act as a



tumor suppressor gene or as an oncogene depending on the cell type and its subcellular localization (Shibata et al. 2003; Pan et al. 2006). The molecular interaction of NHERF1 and PTEN has been described previously (Molina et al. 2012), and NHERF1 is required for 17- $\beta$ -estradiol-increased PTEN expression (Yang et al. 2011).

## Materials and methods

### Tumor bearing mice

Mice lacking Cav-1 and with mammary-specific expression of Her-2/neu were generated by crossing Cav-1 null mice (129/Sv/C57Bl/6) obtained from Dr. T. Kurzchalia (Drab et al. 2001) to mice transgenic for the MMTV-neu oncogene (Guy et al. 1992) as described previously (Sloan et al. 2009). Once the mammary tumors became palpable they were measured weekly using electronic callipers and mice were culled when the primary tumor reached a volume of 1,500 mm<sup>3</sup>. The tumors were removed at autopsy, weighed and fixed in 10 % buffered formalin, dehydrated and embedded in paraffin. A total of 15 animals were examined, the tumors were removed from eight mice with Cav-1<sup>+/+</sup> and from seven mice with Cav-1<sup>-/-</sup>. All procedures were performed in a barrier facility under protocols approved by the Peter MacCallum Animal Experimentation Ethics Committee.

### Immunohistochemistry (IHC)

Hematoxylin and eosin-stained tissue sections (5  $\mu$ m thickness) were used for histopathological studies. Serial 5- $\mu$ m-thick sections were mounted onto 3-aminopropyltriethoxysilane-coated slides (Sigma-Aldrich, St. Louis, MO) for subsequent IHC analysis, which was performed in duplicate (two sections per tumor for each antibody). The primary antibodies used in this study are described in Table 1. An antigen retrieval protocol using heat was used to unmask the antigens (30 min in citrate

buffer 0.01 M, pH6.0). Tissue sections were incubated with the primary antibodies overnight at 4 °C in humidity chambers at the dilutions given in Table 1. A commercial kit to detect mouse and rabbit primary antibodies was used (Dako EnVision system, horseradish peroxidase, diaminobenzidine [DAB]; Dako, Carpinteria, CA). Slides were lightly counterstained with hematoxylin to reveal nuclei, examined and photographed with a Nikon Eclipse E200 microscope (Japan). Non-specific mouse IgG1 antibody and purified rabbit pre-immune serum (Dako, Kingsgrove, NSW, Australia) were used as isotype negative controls. As positive controls we used breast cancer tissue blocks from our tumor bank (Sloan et al. 2009), and the skin tissue of the mice, the optimal dilution of each antibody was tested using these samples. The immunostaining was evaluated in the whole sections with the extent and intensity of immunostaining assessed independently by two experienced researchers blinded to the Cav-1 status of the sample. Disagreements (<10 %, often relating to the level of staining intensity) were resolved by consensus. We used a scoring system reported previously (Gago et al. 1998). Briefly, we used an intensity score: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining and a proportion score: 0 = no staining, 1 = staining in less than 1/10 of the cells, 2 = 1/10 to <1/3 of the cells, 3 = 1/3 to <2/3 of the cells, and 4 = >2/3 of the cells. The total was obtained by combining both intensity and proportion scores, which were reported for membrane, cytoplasmic and nuclear cell compartments. The lack of frozen tissues prevents us from complementing the IHC analysis with more quantitative methods like Western blotting.

### Cells and Western blots

Human MCF-7 breast cancer cells were used to test the specificity of the antibodies used in the present study. Description of the cell growing conditions and the Western blot technique have been presented elsewhere (Cayado-Gutiérrez et al. 2012).

**Table 1** Main characteristics of the antibodies used in the present study

Antibody	Source	Dilution
Her-2/neu	rPAb (Dako, Glostrup, Denmark)	1:200
$\beta$ -Catenin	mMAb (Cat 5H10, Zymed, San Francisco, CA)	1:400
MTA1	rPAb (affinity purified, BL1803; Bethyl, Montgomery, TX)	1:100
PTENa	rPAb (generated against a PTEN synthetic peptide sequence between amino acids 33 to 47 (Perandones et al. 2004)	1:100
PTENb	rPAb (raised against a peptide mapping at the C terminus of PTEN of human origin PTEN C-20 sc-6817-R; Santa Cruz Biotech., Santa Cruz, CA)	1:200
p-Akt	rPAb (generated against an epitope corresponding to amino acids 345–480 of Akt1 of human origin. This antibody detects Akt1, Akt2 and Akt3 (H-136, sc-8312, Santa Cruz).	1:1,000
NHERF1	rPAb (PA 1–090, Affinity BioReagents, Rochford, IL)	1:500

rPAb rabbit polyclonal antibody, mMAb mouse monoclonal antibody

## Statistical analysis

Statistical analyses were completed using the Prism computer program (Graph Pad Software, San Diego, CA); two-tailed paired *t*-test was used for data analysis, a *p* value  $\alpha < 0.05$  was considered significant.

## Results

Co-localization of Her-2/neu and  $\beta$ -catenin in Cav-1<sup>+/+</sup> and Cav-1<sup>-/-</sup> tumors driven by Her-2/neu expression

In a previous examination of these tumors, we reported that Her-2/neu expression was not modified by the absence of Cav-1 (Ciocca et al. 2012). We have now explored the levels of  $\beta$ -

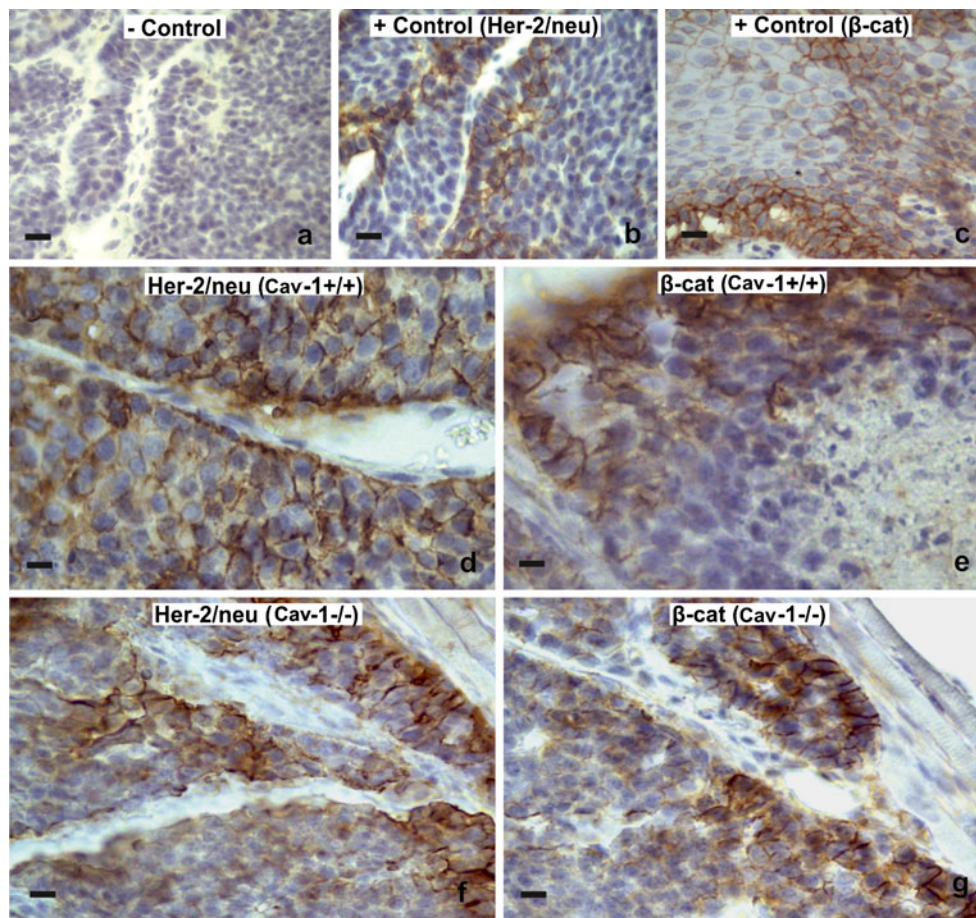
**Table 2** Expression levels of the Her-2/neu,  $\beta$ -catenin, MTA1 and Akt in tumors from Cav-1<sup>+/+</sup> and Cav-1<sup>-/-</sup> mice (semiquantitative evaluation completed on immunostained samples)

Molecule	Score <sup>a</sup> in Cav-1 <sup>+/+</sup>	Score <sup>1</sup> in Cav-1 <sup>-/-</sup>	<i>p</i> value
Her-2/neu	4.8	4.6	NS
$\beta$ -Catenin	4.4	4.16	NS
MTA1	3.72	3.69	NS
p-Akt	2.8	2.37	NS

NS not significant

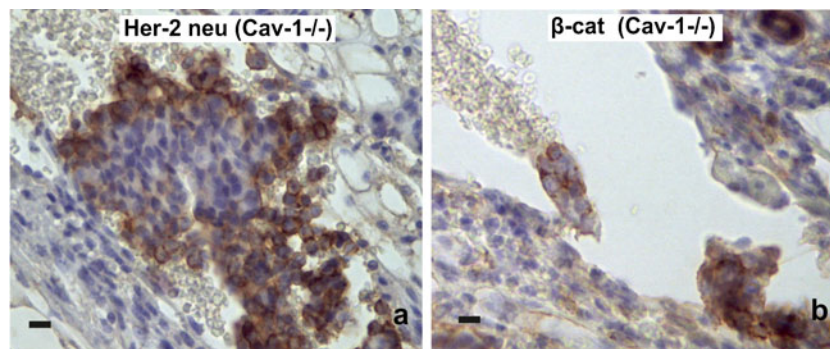
<sup>a</sup> The scoring system used is described in the “Materials and methods” section

catenin and the first notable observation was the co-localization of this protein with Her-2/neu. Both proteins appeared on the



**Fig. 1** Representative photographs of the MMTV-c-neu carcinomas immunostained to reveal Her-2/neu and  $\beta$ -catenin. **a** Negative control using an isotype matched antibody staining of a mammary tumor. Note that in this study we did not have background cross-reaction problems with the immune detection of mouse antigens even when one of the antibodies (anti  $\beta$ -catenin) was of mouse origin (Table 1). **b** Positive control for Her-2/neu in the membrane of the tumor cells. **c** Positive control for  $\beta$ -catenin which appears at the membrane of the epithelial cells in the skin of a mouse. **d** High-power micrograph to reveal Her-2/neu in a tumor from a Cav-1<sup>+/+</sup> transgenic mouse. **e**  $\beta$ -Catenin

immunostaining in a serial section from panel **d**.  $\beta$ -Catenin can be seen at the cell membrane of the tumor cells. **f** High-power micrograph reveals Her-2/neu in a tumor from a Cav-1<sup>-/-</sup> transgenic mouse and a serial section (**g**) shows immunostaining for  $\beta$ -catenin in the Cav-1<sup>-/-</sup> mouse tumor.  $\beta$ -catenin can be seen at the cell membrane of the tumor cells. The images were captured with a Nikon Eclipse E200 microscope ( $\times 10$ – $60$  objectives). The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. Figure magnifications: **a** bar = 100  $\mu$ m, **b** and **c** bar = 70  $\mu$ m, **d**–**e** bar = 25  $\mu$ m, **f**–**g** bar = 35  $\mu$ m



**Fig. 2** Her-2/neu and  $\beta$ -catenin in a mouse carcinoma from an MMTV-c-neu mouse (Cav-1<sup>-/-</sup>). **a** Large cell cluster showing Her-2/neu expression at the periphery of the tumor nests invading a blood vessel. **b** In this serial section, the cell cluster shows  $\beta$ -catenin at the

cell membrane of the tumor cells. The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. Figure magnifications: **a–b** bar = 20  $\mu$ m

surface of the tumor cells, and in the same tumor areas (Fig. 1). The second observation was that, similar to Her-2/neu, the absence of Cav-1 did not significantly change the levels of  $\beta$ -catenin (Table 2). In addition, at a histological level, the tumor cells did not display characteristics of an EMT when Cav-1 was absent (Fig. 1 and the following figures).

The similar levels and localization of Her-2/neu and  $\beta$ -catenin were maintained from the onset of cancer in early hyperplastic lesions, (data not shown) to advanced stage cancer, when the cell clusters were invading the blood vessels (Fig. 2a, b).

MTA1, PTEN, p-Akt and NHERF1 expression in the Cav-1<sup>+/+</sup> and Cav-1<sup>-/-</sup> tumors driven by Her-2/neu expression

MTA1 is induced in human breast cancer cells expressing Her-2/neu (Khaleque et al. 2008). Similarly, in mice MTA1 was present in the nuclei of numerous tumor cells (Fig. 3). However, the absence of Cav-1 did not significantly alter MTA1 expression (Table 2).

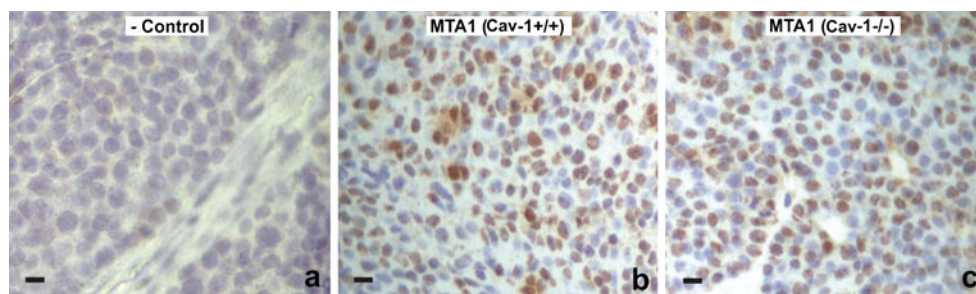
In a previous study, we showed that Hsp27 levels were significantly lower in the Cav-1<sup>-/-</sup> tumors (Ciocca et al. 2012), and recently we have shown that Hsp27 down-regulation increased the expression of PTEN in human MCF-

7 breast cancer cells (Cayado-Gutiérrez et al. 2012). In the present study, PTEN was detected with two different antibodies (Fig. 4a) and significant changes were found in tumors arising from Cav-1 null mice (Fig. 4b). The two different antibodies revealed significantly higher PTEN expression in the tumors grown in the absence of Cav-1, with the protein was mainly localized in the nuclei. “Normal” mammary glands at the periphery of the Cav1 null tumors also showed higher PTEN levels in the nuclei of the luminal epithelial cells.

We also studied two proteins linked functionally to PTEN: p-Akt and NHERF1. P-Akt levels were relatively low in the Her2/neu tumors (Table 2, Fig. 5a). The levels of this protein did not change significantly in either the tumor or in the stroma, when Cav-1 was absent. In contrast, NHERF1 expression levels were increased in the tumors arising in Cav-1 KO mice (Fig. 5b). The protein was present mainly in the nuclei of the tumor cells.

## Discussion

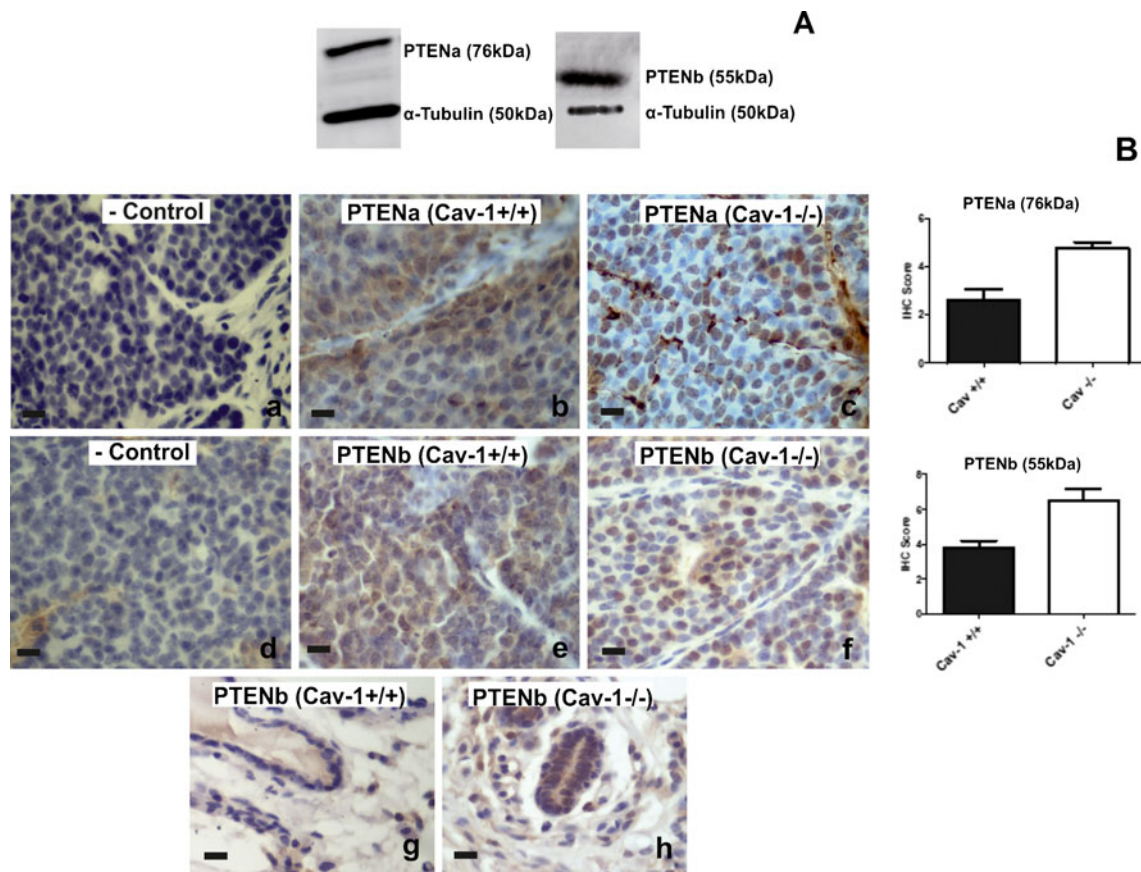
In a previous study using the same transgenic/KO animal model, we reported that the disruption of the *Cav-1* gene



**Fig. 3** MTA1 in Her-2/neu mammary tumors. **a** Negative control using an isotype matched antibody staining of a mammary tumor. **b** MTA1 appears in the nuclei of numerous tumor cells in a Cav-1<sup>+/+</sup> tumor. **c** Similar MTA1 levels can be seen in tumors from Cav-1 null

mice. The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. Figure magnifications: **a–c** bar = 25  $\mu$ m





**Fig. 4** PTEN in MCF-7 human breast cancer cell line (a) and in mouse tumors (b). **a** Two different MW bands were detected using the antibodies, one at 76 kDa (antibody PTENa) and the other at 55 kDa (antibody PTENb) (see “Materials and methods” section and Cayado-Gutiérrez et al. 2012). **b** (a) negative control using an isotype matched antibody staining of a mammary tumor. (b and c) Note the increased expression of PTEN in the Cav-1<sup>-/-</sup> tumor using the anti PTENa antibody (graph,  $p < 0.05$ ). (d) Negative control using an isotype

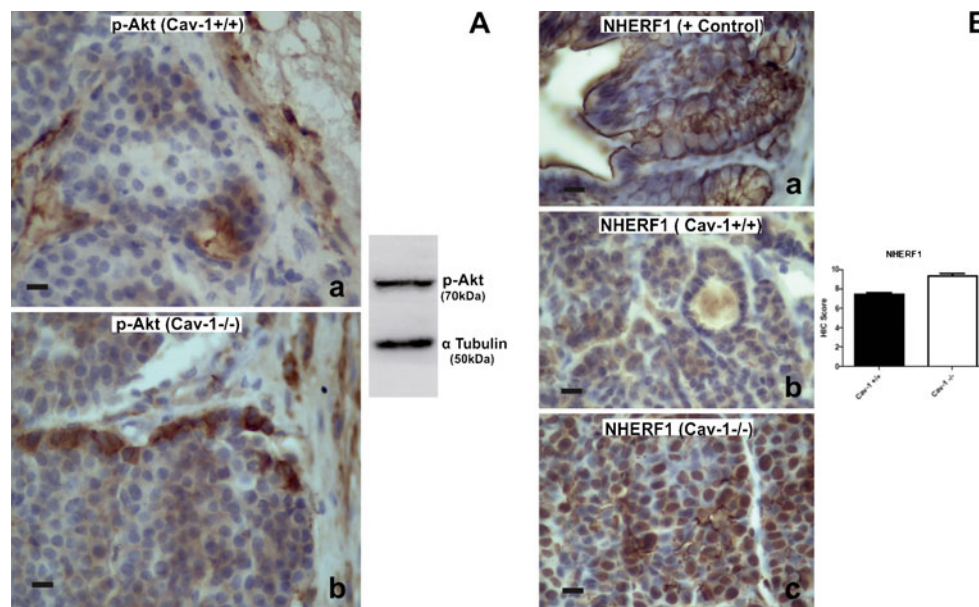
matched antibody staining of a mammary tumor. (e and f) Note again the increased expression of PTEN in the Cav-1<sup>-/-</sup> tumor using the anti PTENb antibody (graph,  $p < 0.05$ ). Relatively “normal” mammary gland also showed more PTEN expression in the Cav-1 KO mouse (g and h, using the anti-PTENb antibody). The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. Figure magnifications, a–f: bar = 70  $\mu$ m, g and h: bar = 50  $\mu$ m

caused alterations in the stress proteins Hsp27, Hsp70 and gp96, as well as in tumor development (Ciocca et al. 2012). We have now advanced these studies by examining other stress-related proteins. The dual functions of Cav-1 on both the cancer epithelium and the cancer stroma are well documented, and there is evidence indicating that Cav-1 depletion causes mislocalization of  $\beta$ -catenin and E-cadherin. These changes are characteristic of EMT in epithelial mammary acini (Mercier and Lisanti 2011). In our study, not only was there no mislocalization of  $\beta$ -catenin in the Cav-1 KO mice, there were also no histological changes indicative of EMT, and  $\beta$ -catenin remained co-expressed with Her-2/neu on the surface of the tumor cells. In other transgenic mouse models (MMTV-Wnt-1 and MMTV-c-Neu), an indispensable interaction between  $\beta$ -catenin and Her-2/neu heterodimers at the cell membrane has been reported, indicating that the interactions between the two molecules is an important signaling event for human breast cancer progression (Schroeder et al. 2002). In contrast, using a similar mouse

tumor model (MMTV/neu) but where the mice expressed a mammary-targeted, mutationally activated HER2/neu allele, cytoplasmic  $\beta$ -catenin was detected in the HER2/neu-induced mouse mammary tumors (Khalil et al. 2012). In addition, the same authors reported in breast cancer patients a correlation between Her-2/neu expression and nucleo/cytoplasmic  $\beta$ -catenin in lymph node-positive carcinomas. Moreover, it is relevant to report here that in a human hypopharyngeal cancer cell line, EGF treatment induced an increased physical interaction between EGFR/ $\beta$ -catenin/caveolin-1 and between E-cadherin/ $\beta$ -catenin/caveolin-1 (Masuelli et al. 2012). These authors also found in human head and neck squamous cell cancer biopsy samples that Cav-1 over-expression appeared associated with simultaneous abnormal expression of the E-cadherin/ $\alpha$ - $\beta$ -catenin complex and with multiple ErbB receptors resulting in a more aggressive phenotype.

Several molecules are influenced when Her-2/neu is activated. Two of them, MTA1 and heat shock factor 1, are strongly





**Fig. 5** Representative photographs of the MMTV-c-neu transgenic carcinomas immunostained to reveal p-Akt and NHERF1. **a** Western blot showing that the anti p-Akt antibody detected a 70-kDaMW band (MCF-7 cells); the same paper was incubated with anti-tubulin (lower band). There is a relatively low p-Akt expression in the tumors (*a* and *b*). In one of the tumors grown in a Cav-1<sup>-/-</sup> mouse (*b*), cells at the periphery of a tumor nest shows more p-Akt, but this did not change the overall scores (Table 2). **b** Western blot is not shown here because the specificity of the

antibody was demonstrated in a previous study (Cuello-Carrión et al. 2010). (*a*) Positive control using colon, the immunoreaction can be seen at the cell surface and in some nuclei. Note the increased expression of NHERF1 in the Cav-1<sup>-/-</sup> tumors, mainly in the nuclei (*b* and *c*,  $p < 0.05$ ). The positive immunoreactivity appears as brown deposits, and the slides were lightly counterstained with hematoxylin to reveal nuclei. Figure magnifications, bar = 25  $\mu$ m

induced and form complexes (both together and with other proteins) that participate in the repression of estrogen-dependent transcription (Khaleque et al. 2008). In the mouse model used in this study, the tumor cells did not express estrogen receptor alpha or beta and although heat shock factor 1 was present, the levels were not altered by the absence of Cav-1 (Ciocca et al. 2012). Likewise, the tumors expressed MTA1, but once again the levels were not affected by the Cav-1 status.

PTEN helps to maintain the balance of tyrosine phosphorylation of cellular proteins implicated in normal and pathological signaling pathways. Most protein tyrosine phosphatases, including PTEN, contain at least one Cav-1-binding motif that results in the formation of molecular complexes (Caselli et al. 2002). Therefore, at least on the cell surface and in cytoplasmic membranes, Cav-1 is associated with PTEN (Caselli et al. 2002). In our study, Cav-1 was not detected by IHC in the cancer cells from the Cav-1 wild type tumors even though the stroma was positive for Cav-1 (Ciocca et al. 2012). However, PTEN was present in both the Cav-1 wild type and null tumors. Therefore, although Cav-1 interacts with PTEN, the tumor cells can express PTEN in the absence of Cav-1 and we found that the absence of Cav-1 in the stroma significantly induced the tumor cells to express more PTEN. The tumors also had lower Hsp27 levels (Ciocca et al. 2012). These findings support our recent study where we showed the down regulation of Hsp27 to induce over-expression of PTEN (Cayado-Gutiérrez et al. 2012).

The increase in PTEN was seen mainly in the nuclei of the epithelial cancer cells. Nuclear PTEN has been associated with regulation of the expression of many genes, and although the mechanism of this regulation remains unclear, it has been postulated that PTEN may have a non-phosphatase function in the nucleus (Moncalero et al. 2011). In the present study we did not find significant changes in p-Akt levels when Cav-1 was absent, which validates a non-phosphatase role of PTEN. In a previous study, an increase in PTEN was accompanied by a decrease in p-Akt (Cayado-Gutiérrez et al. 2012). Aldred et al. (2003) reported that nuclear PTEN is found predominantly in normal thyroid glands but not in thyroid carcinomas, where the PTEN levels are low and located in the cytoplasm. Several reports indicate that more aggressive cancers are linked to the absence of nuclear PTEN (Whiteman et al. 2002; Zhou et al. 2002; Tsutsui et al. 2005; Fridberg et al. 2007). Of interest in our study is that the group of animals with mammary adenocarcinomas grown in Cav-1 null mice showed a trend towards a lower incidence of visible lung metastases than the tumors grown in Cav-1<sup>+/+</sup> mice (20 % vs. 28 %; Sloan et al. 2009). In contrast, the absence of Cav-1 reduced the amount of apoptosis and caused an earlier onset of mammary tumors, while in breast cancer patients the absence of Cav-1 in the stroma has been related with a more aggressive phenotype. Therefore, future studies are required to clarify the apparent discrepancies between the transgenic mouse model and the cancer patients.

In this study, as was the case with PTEN, NHERF1 was also elevated in tumor cells from Cav-1 null mice, and both proteins appeared mainly in the nucleus. In the cytoplasm, NHERF proteins recruit PTEN to PDGFR to restrict the activation of the PI3K (Takahashi et al. 2006). NHERF1 also complexes directly (through the PDZ2 domain) with  $\beta$ -catenin, and this association is important in maintaining  $\beta$ -catenin localization at the cell surface (Kreimann et al. 2007). Our study revealed that these three proteins can follow different cellular localization routes, since even when PTEN and NHERF1 are in the nucleus,  $\beta$ -catenin can still be on the cell surface. The question beckons, what is NHERF1 doing in the nucleus? It seems that NHERF1 can stabilize  $\beta$ -catenin/TCF-1 complexes, which causes  $\beta$ -catenin to bind to dominant negative TCF-1. This in turn results in enhanced Wnt/ $\beta$ -catenin signaling events that influence the transcription of downstream oncogenes (c-Myc and cyclin D1) (Lin et al. 2012). These authors also reported that nuclear NHERF1 is related to poor clinical outcome in colon cancer. In our study, tumor onset was more rapid in mice lacking Cav-1 and these animals had a shorter overall survival (Sloan et al. 2009). Additional experiments should be completed to clarify the role(s) of nuclear NHERF1 (independent of  $\beta$ -catenin).

Taken together, the data obtained from the MMTV-neu model are consistent with a role for Cav-1 in stromal cells in breast cancer by modulating the expression and localization of proteins implicated in tumor progression.

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